

Next-generation sequencing showing potential leachate influence on bacterial communities around a landfill in China

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ABSTRACT

The impact of contaminated leachate on groundwater from landfills is well known but specific effects on bacterial consortia are less well-studied. Bacterial communities in landfill and an urban site located in Suzhou, China were studied using Illumina high-throughput sequencing. A total number of 153944 good quality reads were produced and sequences assigned to 6388 operational taxonomic units (OTUs). Bacterial consortia consisted of up to 16 phyla including *Proteobacteria* (31.9 to 94.9% at landfill, 25.1 to 43.3% at urban sites), *Actinobacteria* (0 to 28.7% at landfill, 9.9 to 34.3% at urban sites), *Bacteroidetes* (1.4 to 25.6% at landfill, 5.6 to 7.8% at urban sites), *Chloroflexi* (0.4 to 26.5% at urban sites only) and unclassified bacteria. *Pseudomonas* was the dominant (67-93%) genus in landfill leachate. Arsenic concentrations in landfill raw leachate (RL) (1.11×10^3 $\mu\text{g/L}$) and fresh leachate (FL2) (1.78×10^3 $\mu\text{g/L}$), and mercury concentrations in RL (10.9 $\mu\text{g/L}$) and FL2 (7.37 $\mu\text{g/L}$) were higher than Chinese State Environmental Protection Administration (SEPA) standards for leachate in landfills. Shannon diversity index and Chao 1 richness estimate showed RL and FL2 lacked richness and diversity when compared with other samples. This is consistent with stresses imposed by elevated arsenic and mercury and has implications for ecological site remediation by bioremediation or natural attenuation.

Keywords Landfill, leachate, bacterial diversity, *Pseudomonas*, Arsenic.

INTRODUCTION

Municipal landfill waste compositions can range from food wastes to high-strength detergents, solvents and pharmacological products comprising a broad spectrum of xenobiotic and recalcitrant toxic compounds with potential harmful ecological impacts (Köchling et al., 2015, Song et al., 2015a). Although modern landfills in well-regulated economies are highly engineered and monitored, older or informal (unplanned, uncontrolled) landfills worldwide are sources of leachate which, unless correctly collected and treated, can cause serious reductions in the quality of water bodies and groundwater sources (Li et al., 2014, Zhang et al., 2013a). Previous studies have indicated a diverse range of heavy metal concentrations in leachates (Song et al., 2015b, Zhang et al., 2013a). Heavy metals have been previously shown to directly influence the bacterial community composition of various environments (Muller et al., 2001, Vishnivetskaya et al., 2011, Sandaa et al., 1999, Mor et al., 2006, Yao et al., 2017). Long term studies have shown a strong influence of mercury towards the bacterial community of a river basin and soil (Muller et al., 2001).

To study complex microbial ecosystems such as leachate, molecular techniques have several advantages over culture-based techniques as they allow the analysis of uncultured organisms and provide higher resolution measurements closer to the complete microbial profile (Staley et al., 2011). Analysing the microbial community around a landfill can potentially determine whether the leachate is being transported through the landfill liner into the natural soil and groundwater, via changes in the diversity and composition of bacterial consortia as different species are more or less tolerant of elevated pollutant concentrations (Wang et al., 2017, El-Salam and Abu-Zuid, 2015, Vukanti et al., 2009).

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70 Previous studies on heavy metal influence towards microbial communities were performed using
71 PCR-DGGE and GS 454 FLX pyrosequencing (Muller et al., 2001, Yao et al., 2017,
72 Vishnivetskaya et al., 2011). Next generation sequencing (NGS) methods can assist in the
73 identification of very rare taxa in the landfill samples (Köchling et al., 2015, Song et al., 2015a).
74 NGS provides efficient, multiple level details of the operational taxonomical units (OTUs),
75 richness and diversity, so it can be used to identify both similarities and differences between
76 sites. Furthermore, the rapidity and portability of NGS methods and apparatus, for example,
77 Nanopore (Oxford Nanopore Technologies, Oxford, UK) mean that sequencing of microbial
78 consortia now presents a potentially rapid, low-cost option for the detection of leachate impacts
79 on natural groundwater consortia and hence mapping of contaminant plumes based on
80 ecological, rather than chemical, indicators (Brown et al., 2017).

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82 Understanding the environmental conditions and bacterial community is of utmost importance
83 when it comes to cleaning up the contaminants by employing techniques such as biodegradation.
84 It is a microbial process that degrade contaminants found in the environment. Over the past 20
85 years, in-situ biodegradation has successfully been applied to various environments with
86 different level of degrading abilities depending on the bacteria (Meckenstock et al., 2015). The
87 process requires careful identification of the degrading bacteria prior to implementation.
88 Generally, constant monitoring of the microbial activity is also required to ensure constant and
89 consistent microbial activity over time. For example, Adetutu et al. (2015) utilised biostimulation
90 (BS), biostimulation-bioaugmentation (BS-BA) and monitored natural attenuation (MNA)
91 approaches to bioremediate groundwater polluted with trichloroethene (TCE). Next-generation

sequencing was an effective technique to study the microbial community dynamics throughout while performing the dechlorination process.

In the present work, we investigated the potential for NGS to identify potential impacts on soil and groundwater bacterial communities due to heavy metal-rich landfill leachate in a conurbation in Suzhou, Jiangsu province, China. The objectives of this study were i) to characterize the composition of the bacterial communities of a selected landfill (leachate, soil and groundwater) and a non-landfill site in same conurbation, hereby referred to as “urban” (soil and groundwater); ii) to compare the unique and dominant bacterial taxa among the landfill and urban samples; and iii) to investigate and compare the bacterial diversity and heavy metal concentration of the soil and groundwater samples from a landfill and urban site. The study not only adds to the knowledge in respect of leachate impacts on subsurface consortia under urban areas, but assesses the potential of NGS for rapid monitoring of environmental impacts from landfills, and has implications for the design and implementation of biological remediation options such as natural attenuation or *in situ* microbially-induced carbonate precipitation.

MATERIALS AND METHODS

Sample locations

The selected landfill (located at 31°14'18.31"N 120°33'3.09"E) began operation in 1993 and receives about 1,500 tons/day of household wastes and industrial wastes from the Suzhou conurbation. A new landfill was constructed in 2006 on the surface of the older landfill (Rong et al., 2011). The urban site samples were collected from an area that was previously used for agriculture prior to reclamation for industrial development. The two sites are approximately 27

km from each other. The two sites are approximately 27 km from each other. Suzhou is situated on top of a 200 m deep sequence of Quaternary sediments. The depth of drift reduces to 0m directly to the West and South West of the City (Jiangsu Provincial Bureau of Geological and Mineral Exploration, 1984). At depth the bedrock is composed of Devonian quartzite and shales of the Wutong Formation, the sandstones shales and quartzites of the Maoshan Group and zones of Carboniferous limestone (the karstic features of which are known commercially as Taihu Stone, exposed at Dongting Mountain and in Linwu Cave) which forms the hills to the south and west of the city. This sequence is intruded by the Suzhou Granite which is exposed to the West of the city centre. The variable erosive bedrock surface, has been infilled by alluvial and lacustrine sediments of the lower flood plains of the Yangtze River. The subsurface materials vary from clays to silty sands (Shi et al., 2012). The structure of the quaternary strata below ground varies at the very large scale, due to the movement of the rivers and changes in the extent and location of the lakes with time. However, the extent of variation has been limited by the volume of materials being deposited within a geologically short period of time. Some of the silty/sandy subsurface zones are a result of reworking of loess by the Yangtze River. The silty sands have sufficient porosity to act as aquifer materials (Ma et al., 2011). Pumping works from these aquifers have caused the collapse of their porous structure resulting in approximately 1 m of settlement across the region increasing to 1.4m towards city centres, and reducing to 0m towards the locations of large permanent lakes (Shi et al., 2012). Details regarding Suzhou landfill construction and waste were briefly discussed by Rong et al. (2011).

The landfill sampling comprised of two leachates, soil from three different locations around the landfill (samples LS1, LS2 and LS3) and one groundwater from the landfill monitoring well

(samples BHGW) (Table 1). Leachate samples were either fresh (FL2, collected from an outlet pipe that runs beneath the landfill) or raw leachate (RL, sampled from a leachate pond). Soil samples were collected using a Spiral auger at 30cm depth. The first soil location was near the leachate pond; the second was close to agricultural land on the boundary of the site; and the third soil location was close to the groundwater monitoring borehole. The groundwater was collected at an approximate depth of 4 meters using a hand-held slow flow peristaltic pump. The samples were collected from well below the groundwater surface such that any residual floating matter would not be collected. Groundwater and leachate were collected in sterile high density polyethylene plastic bottles and soil samples were collected in a sterile plastic zip lock bags and transported to the laboratory under ambient temperature conditions, then stored in a cold room (4°C) prior to analysis.

To contrast the bacterial community from the landfill, soil (samples USS1 and USSur1) and groundwater (samples USGW) samples were collected from the urban site. Two samples from the two different locations in an urban area were selected for the soil sampling which were 200 meters apart. The groundwater borehole was chosen for the groundwater sampling. Ground water was collected at a depth of 4 meters. The first location of the soil sampling was located closer to the urban site groundwater and the second location of the soil sample was an isolated location.

Physicochemical analysis of soil and water samples

The following heavy metals were analysed for all samples: mercury (Hg), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn) and chromium (Cr). The heavy metals were analyzed at Tsingcheng Environment Company in Suzhou, China. Mercury and arsenic were analysed using Atomic Fluorescence Spectroscopy (AFS 2100, Haiguang Instruments Co. Ltd); zinc, lead and

copper were analysed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP 710, Agilent Technologies); cadmium was analysed using graphite furnace-Atomic Absorption Spectroscopy (240Z, Agilent technologies) and chromium was analysed using Flame-Atomic Absorption Spectroscopy (ICP 710, Agilent technologies). The pH of soil, groundwater and leachate samples was measured using a Suntex[®] TS 3000 pH/Temp portable probe in the Department of Environmental Science at XJTLU. The samples were stored at +4°C prior to analysis.

Preparation and extraction of DNA from soil, leachate and groundwater samples

Preparation of samples for DNA extraction

One liter of groundwater was filtered on a 0.22 µm pore size polycarbonate membrane filter (Millipore, USA) using a vacuum pump. Samples were filtered and the filters were placed in sterile Petri dishes and stored at -20°C until they were used for DNA extraction. Due to the nature of the sample (high turbidity), 50 ml of leachate was centrifuged at 5000 rpm for 5 minutes and both the pellet and the supernatant were collected. The supernatant was filtered in a 0.22 µm membrane filter (Millipore, USA) and both pellet and membrane filter were used for DNA extraction. Soil samples were weighed (0.25 g) and used for DNA extraction.

DNA extraction

The genomic DNA from all the samples was extracted using a commercial DNA extraction Kit (MO BIO Power soil[®] DNA kit, USA) according to the manufacturer protocol. 50 µl of elution buffer was used to elute the DNA samples and these were frozen at -20 °C until further processing for bacterial community analysis. The DNA was quantified using Nanodrop (Thermo Scientific, Waltham, MA, USA) and examined by agarose gel electrophoresis (1% w/v).

Bacterial community analysis by next-generation sequencing

The bacterial diversity and community composition of soil, leachate and groundwater samples were studied by NGS using the Illumina MiseqPE250 platform. NGS was carried out at Shanghai Majorbio Pharmaceutical Technology Limited, China. 16S rRNA genes (V4 region) were amplified by PCR using 515F (5'barcoded GTGCCAGCMGCCGCGG3') and 806R (5'GGACTACHVGGGTWTCTAAT3') primer sets. PCR reactions contained in 20 µl: 4 µl of 5× FastPfu Buffer, 2 µl of 2.5 mM dNTPs, 0.8µl of forward and revers primers (5 µM), 0.4 µl of FastPfu polymerase, 10 ng of template DNA and DD water up to 20 µl. PCR conditions: a ABI GenAmp 9700 thermocycler was used. Initial denaturation 3 minutes at 95°C was followed by 28 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C; final extension was carried out at 72°C for 10 min. The purified amplicons were pooled and sequenced on an Illumina MiSeq platform. Chimeric sequences were removed and the operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (Edgar, 2013). The phylogenetic affiliation of each 16S rRNA sequence was analysed by RDP classifier against the SILVA data base (Pruesse et al., 2007). The sequences were submitted to National Centre for Biotechnological Information (NCBI) Short Read Archive (SRA) database under the accession numbers SAMN06339740 to SAMN06339748.

Data analyses

The diversity within each sample (alpha diversity) was calculated by Shannon (H') and Simpson (D) diversity indices, abundance based coverage estimator (ACE) and Chao 1 richness estimator using MOTHUR (<http://www.mothur.org>). The diversity between samples were compared (beta diversity) by non-metric multidimensional scaling (NMDS) and cluster analysis by using QIIME. The relationship between the environmental parameters (pH and heavy metals) and bacterial

community was assessed by redundancy analysis (RDA) or canonical correspondence analysis (CCA) by using R language vegan package.

RESULTS

pH and heavy metals

Tables 2 and 3 show that the soil samples from the landfill and urban site were slightly acidic while landfill groundwater (BHGW), raw leachate (RL) and fresh leachate (FL2) sample were alkaline. To ensure accuracy in the results, two samples were collected for the landfill sites. The two readings labelled as ⁽¹⁾ and ⁽²⁾ were taken from the same pool at slightly different location and interval. The Arsenic concentrations in RL and FL2 were 11.1-12.3 to 17.8-18.4 times higher than the Chinese SEPA guideline concentration value for landfill of 100 µg/L – Class V (Yang et al., 2008). Mercury concentrations were an order of magnitude higher in RL and FL2 samples and in the BHGW (landfill groundwater) than the Chinese SEPA guideline values (Yang et al., 2008). Heavy metal concentrations of the soil samples from the landfill were within the guideline range (Table 3). The As concentration of urban site soil 1 and 2 (USS1 and USSUR1) was at the threshold tolerance value of the guideline range. The heavy metal concentration of Hg in BHGW was found to be 340 times higher than USGW.

Bacterial diversity

Table 4 shows the number of reads obtained from the landfill samples varied from 13611 to 20464 and in urban site, it ranged from 14015 to 22643. The maximum reads obtained from LS3 and lowest from LS2 in the landfill environment. In urban site, USSUR1 had the lowest reads compared to other urban samples. OTU values ranged from 139 to 1018 for the landfill samples compared to 168 to 1167 in the urban site samples. FL2 had the lowest number and BHGW had the highest number of OTUs. In the urban site, USGW had the lowest OTU read compared to

USS1 which had the highest OTU read of 1224. The bacterial richness and diversity (Shannon H' index) of the urban soil samples (USS1 and USSUR1) were the highest of all the samples. Species diversity estimates obtained for the abundance-based coverage estimators (ACE) and the Chao1 index was higher in the urban site soil samples when compared to the landfill soil samples, despite As concentrations an order of magnitude higher in the urban site soil samples than in the landfill soil samples. Furthermore, the landfill groundwater (BHGW) had more bacterial diversity than the urban groundwater (USGW) by every metric despite the Hg concentration in BHGW being more than 340 times higher than USGW (Table 2).

Bacterial community structure

Figure 1 shows the bacterial community composition at phylum level in both landfill and urban site samples. Among all the phyla, only *Proteobacteria* and *Bacteroidetes* were found to be present in all the samples. The phylum *Proteobacteria* was dominant in all the samples from landfill site with their abundance ranging from 31.4% to 94.9% in the landfill samples. Across the urban site, their abundance ranged from 25.1% to 43.3% with USGW possessing a lower abundance compared to the USS1 and USSUR1. *Bacteroidetes* abundance ranged from 1.42% to 25.64% among the landfill samples with FL2 having the lowest abundance and LS2 the highest. In the urban site, samples they ranged from 5.69% to 7.86% in abundance with USGW having the higher presence of *Bacteroidetes*. Members of phylum *Actinobacteria* were found in all the samples except the leachate samples. The relative abundance of *Actinobacteria* ranged from 12.6 % to 28.6% and from 9.9% to 34.3% for the landfill site and urban site, respectively. USGW was again found to be higher for *Actinobacteria*. *Chlamydiae* was only found in USGW at 24.1%. *Firmicutes* and *Thermotogae* were only found in the RL sample with 6.4% and 8.2% abundance, respectively.

Figure 2 shows that at the order level, *Pseudomonadales* and *Sphingobacteriales* were present in all samples. *Pseudomonadales* were dominant in the landfill samples at RL (69.96 %), FL2 (92.97 %), LS2 (25.29 %) and LS3 (16.11 %). In LS2 and LS3, either *Xanthomonadales* (11.04% and 14.09%) or *Flavobacteriales* (20.88% and 10.55%) were the second or third dominant orders observed. However, in USGW samples, *Frankiales* (34.06%) and *Chlamydiales* (24.09%) were dominant and their abundance was either <1% or absent in other samples from both sites. *Sphingobacteriales* were found to be the second dominant order at 8.5% for BHGW and 7.81% for USGW. *Flavobacteriales* were present in higher percentages in LS2 (20.88%) and LS3 (10.55%) but their abundance were found to be less than <2% in other samples.

At genus level, the bacterial communities from the two sites were more diverse and unique. Figure 3a shows that *Pseudomonas* was the most dominant genus observed in FL2 and RL with a relative abundance of 92.9 and 69.9%, respectively. This genus was also dominant in LS2 and LS3 but their relative abundance was less (16-25%) as compared to leachate samples. *Sphingomonas* (6.5%) was found to be dominant in BHGW. In contrast the urban site samples (Figure 3b) show *Sporichthyaceae_unclassified* (34%) to be dominant followed by *Candidatus_Rhabdochlamydia* (24%) and *Sediminibacterium* (5.83%) in USGW sample. *Thiobacillus*, *Anaerolineaceae_uncultured* and *Nitrosomonadaceae_uncultured* were dominant in USS1 and USSUR1 samples.

Cluster analysis and NMDS was performed on the landfill and urban site samples (Fig. 4a, 4b, 5a, 5b). Fig 4a indicates a high level of similarity among the LS1, LS2 and LS3, BHGW, USS1

and USSUR1 samples. RL, FL2 and USGW are shown to be unique compared to the rest of the samples. Cluster analysis shown in Fig 5a and 5b support the results observed for RL, FL2 and USGW in Fig 4a. Fig 4b shows the least level of similarity observed among RL, FL2, LS1, LS2, LS3, USS1 and USSUR1 samples.

To study the relationship between environmental parameters and bacterial community composition, both multivariate redundancy analysis (RDA) and canonical correspondence analysis (CCA) were performed and compared since the length of the first axis gradient were between 3.0 and 4.0. Fig. 6 shows the RDA plot of the influence of As, Pb, Hg and pH on the soil samples from the different locations. The USS1 and USSUR1 samples were mainly correlated with the As and Pb content in the soil. The LS3 samples exhibited the reverse pattern and were correlated with the pH and Hg concentration in the soil. Canonical correspondence analysis (CCA) was performed to determine the possible linkages between the bacterial communities and environmental parameters by examining the leachate and groundwater samples. Canonical correspondence analysis (CCA) showed a negative correlation between As, pH, Hg and the bacterial community of the samples, indicating that they had the biggest impacts on the bacterial community of these samples (Fig. 7). Arsenic was the major factor that negatively correlated with bacterial communities from FL2 and RL samples. CCA identified both pH and heavy metals in the samples as a major environmental factor in affecting bacterial communities.

DISCUSSION

Comparison of pH and heavy metals between sites

The pH of leachate samples RL and FL2 were 7.78 and 8.12, respectively (Table 2). This range of pH has been reported in other landfill leachate studies conducted in China (Song et al., 2015a,

Song et al., 2015b, Li et al., 2014). Since this landfill has an onsite incinerator, the alkaline pH could be attributed to the disposal of ash in the landfill. The pH of BHGW and urban site groundwater (USGW) was also alkaline at 8.2 and 7.75, respectively (Table 2). The pH values of landfill and urban site soil were between 6.6 and 7.1 which indicate that the samples are slightly more acidic in nature than the natural groundwater (Table 3). The pH values of the soil are not surprising given the sites were previously used as agricultural lands (Zou et al., 2014) and the regional presence of limestone formations (Jiangsu Provincial Bureau of Geological and Mineral Exploration, 1984).

The heavy metal concentrations for As and Hg were above the guidelines range in both leachate samples (Table 2). These hazardous ranges of As and Hg could be due to the solid waste decomposition (mostly from waste water and MSW) and indicates the age of the landfill (more than 10 years old) (Zhang et al., 2013b, Huang et al., 2013, Huang et al., 2003). The Hg level in BHGW was 340 times higher when compared with USGW, indicating a possible percolation of mercury from the landfill leachate to landfill groundwater. Very low concentrations in LS1, LS2 & LS3 indicating Hg-bearing leachate and groundwater are not interacting with the soils. On this chemical evidence, it might be concluded that at this site, the near surface environment around the landfill remains relatively uncontaminated and leachate was not percolating directly to the groundwater below the water table (Roling et al., 2001) (Wang et al., 2011).

The concentration of As in RL & FL2 was very high in comparison to other landfills in Jiangsu province which was between 0.03 to 0.113 mg/L. (Yang et al., 2008). Given that both sites were agricultural land prior to rapid urbanisation in the late 20th century, agri-chemical residues

within the soil at USS1 & USSUR1 could explain the elevated arsenic levels (Zou et al., 2014). The remaining heavy metals were analyzed from both sites and are typical of soils in urban contexts subject to uncontrolled disposal of consumer and industrial chemicals, road runoff and deposition of airborne pollutants (Mor et al., 2006). (Wijesekara et al., 2014). This context of high background contamination presents the key challenge for both chemical and microbiological investigation of leachate impacts.

Analysis of bacterial community structure in landfill

Comparison OTU and community composition among samples

Figs. 4 and 5 shows OTU based NMDS and cluster analysis plots which demonstrate the level of similarity among the samples from both sites. When aggregated together, similarity between landfill soil samples (LSO) and urban site soil samples (USO) was high when compared against the similarity between groundwater samples from both sites (Fig. 4a). Landfill groundwater (BHW) consortia were also closely similar with the soil samples. The reason behind the low similarity between the groundwater samples could be due to the poor diversity and richness of the urban groundwater (USGW) (Table 3). It is also clear that the bacterial communities in the raw and fresh leachate were markedly distinct from any of the soil or groundwater communities; this is evident at both genus and order level (Figs. 2 and 3). On the basis of bacterial community analysis, the dramatic differences between leachate and environmental samples offer the potential for fingerprinting the presence of leachate contamination through identification of leachate-specific DNA in environmental samples. Although such detailed mapping was not possible in this study, we note that all three landfill soil samples contained *Pseudomonas*, in common with the leachate samples, which was not present in soils or groundwater from non-

landfill locations. This may indicate surface or in-soil transport of leachates not evident from the heavy metals analysis.

Dominant phyla and genera in both sites

Leachate samples RL and FL2 had the least diverse phyla detection, in contrast to other landfill leachate studies (Song et al., 2015a, Wang et al., 2017). The high concentration of As and Hg in RL and FL2 could have inhibited the growth of other phyla, whereas *Pseudomonas* spp. have recently been identified as key members of arsenotrophic consortia in contaminated groundwater environments in Bangladesh (Sultana et al., 2017). The low diversity in leachate samples, compared with samples taken from within the landfill (e.g., (Wang et al., 2017) may also be due to the concentration of landfill microbiota within surface-attached biofilms rather than in mobile planktonic forms (Costerton and Wilson, 2004). Landfill and urban site soil and groundwater samples shared most of the phyla except for *Chlamydiae*; which was only found in USGW. As far as we are aware, this is the first study to observe significant presence of *Chlamydiae* in urban groundwater microbial consortia; interestingly, given the high levels of lead and zinc in the urban soils, the phyla has previously been isolated in groundwater samples affected by lead-mine tailings (Zhang et al., 2008) .

Proteobacteria were most dominantly found in leachate samples from landfills (Song et al., 2015a, Song et al., 2015b) and aquifer sediments (Wan et al., 2012). It has been reported that members of *Proteobacteria* involved in the degradation of aromatic oils such as polycyclic aromatic hydrocarbons (Vukanti et al., 2009). These bacteria have been found to lose dominance in older leachate samples (Köchling et al., 2015) and they were detected at highly abundant levels in aged refuse from Shanghai landfills (Xie et al., 2012). *Actinobacteria* was found in the

soil and groundwater samples from both sites but not in the leachate samples. This was not expected as *Actinobacteria* has previously been found in leachate samples (Vukanti et al., 2009). The high arsenic and mercury concentrations of leachate could perhaps have restricted their growth. *Actinobacteria* are responsible for organic matter degradation contributing to carbon turnover (Song et al., 2015b). Since landfills receive waste ranging from households to industries, the amount of organic matter present in the soil could be a reason behind their presence in landfill soil compared to urban site soil. *Bacteroidetes* was observed in abundance at BHGW being twice as much as USGW. While LS2 & LS3 had three times the dominance as USS1 & USSUR1 which could possibly indicate early stages of organic matter degradation within the landfill samples as they commonly contain more soluble and easily degradable material (Schmidtova and Baldwin, 2011). *Bacteroidetes* tend to become more dominant than Proteobacteria as the waste in the landfill ages (Köchling et al., 2015). *Firmicutes* was only found to be dominant in the leachate samples which suggest that they are able to withstand and survive the toxic heavy metal concentrations found in the leachate. They have also been found in other toxic chemical environments such as sewers and drainage (Rodrigues et al., 2014). Environmental factors may have fundamental impacts on the structure and function diversity of bacterial communities in landfill. Analysis from RDA showed that LS1, LS2, LS3 and BHGW were not influenced by pH and heavy metals, where USS1 and USur1 were shown to be lightly influenced by As and Pb. In this study, analysis from CCA has shown that higher concentrations of As and Hg influence the bacterial community of leachate. pH was also shown to significantly influence the bacterial community of leachate. The findings from this paper are consistent with previous results that show that heavy metals influence the bacterial community of landfill (Yao et al., 2017).

Potential of NGS for fingerprinting leachate interactions with soil and groundwater

In this study, Illumina MiSeq technique was used to investigate the bacterial community in samples collected from landfill and urban sites. Bacterial richness and abundance were found to vary significantly among the landfill and urban site samples. Further bacterial analysis revealed lack of diversity in leachate samples when compared with soil and groundwater samples. OTU data from NGS could be used in mapping the interactions between the samples at a site. In our study, OTU data helped in understanding the similarity among the samples from both sites. More studies are now being published using MiSeq methodology since it offers high-resolution microbial community data which helps us in understanding the influence of external factors such as heavy metals towards soil and groundwater microbial consortia. Further study needs to be conducted to understand the long term effects of leachate interactions with soil and groundwater in a landfill to observe the changes in microbial community.

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Conflict of Interest

The authors mentioned in this paper have no conflict of interest regarding the paper's content and submission.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Draft

References

- ADETUTU, E. M., GUNDRY, T. D., PATIL, S. S., GOLNESHIN, A., ADIGUN, J., BHASKARLA, V., ALEER, S., SHAHSAVARI, E., ROSS, E. & BALL, A. S. 2015. Exploiting the intrinsic microbial degradative potential for field-based in situ dechlorination of trichloroethene contaminated groundwater. *J Haz Mat*, 300, 48-57.
- BROWN, B. L., WATSON, M., MINOT, S. S., RIVERA, M. C. & FRANKLIN, R. B. 2017. MinION™ nanopore sequencing of environmental metagenomes: a synthetic approach. *GigaScience*, 6, 1-10.
- COSTERTON, W. J. & WILSON, M. 2004. Introducing Biofilm. *Biofilms*, 1, 1-4.
- EDGAR, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*, 10, 996-1001.
- EL-SALAM, M. M. A. & ABU-ZUID, G. I. 2015. Impact of landfill leachate on the groundwater quality: A case study in Egypt. *J Adv res*, 6, 579-586.
- HUANG, K., GUO, J., LIN, K. F., ZHOU, X. Y., WANG, J. X., ZHOU, P., XU, F. & ZHANG, M. L. 2013. Distribution and temporal trend of polybrominated diphenyl ethers in one Shanghai municipal landfill, China. *Environ Sci Pollut Res Int*, 20, 5299-5308.
- HUANG, L.-N., CHEN, Y.-Q., ZHOU, H., LUO, S., LAN, C.-Y. & QU, L.-H. 2003. Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill. *FEMS microbiol ecol*, 46, 171-177.
- JIANGSU PROVINCIAL BUREAU OF GEOLOGICAL AND MINERAL EXPLORATION. 1984. *Geological map of Jiangsu Province and Shanghai Municipality, PRC*.
- KÖCHLING, T., SANZ, J. L., GAVAZZA, S. & FLORENCIO, L. 2015. Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. *Appl Microbiol and Biotechnol*, 99, 5657-5668.
- LI, Y., LI, J. & DENG, C. 2014. Occurrence, characteristics and leakage of polybrominated diphenyl ethers in leachate from municipal solid waste landfills in China. *Environ Pollut*, 184, 94-100.
- MA, F. S., YANG, Y. S., YUAN, R. M., YAO, B. K. & GUO, J. 2011. Effect of regional land subsidence on engineering structures: a case study of the 6 km long Su-tong Yantze River Bridge. *Bulletin of Engineering Geology and the Environment*, 70, 449-459.
- MECKENSTOCK, R. U., ELSNER, M., GRIEBLER, C., LUEDERS, T., STUMPP, C., AAMAND, J., AGATHOS, S. N., ALBRECHTSEN, H.-J., BASTIAENS, L., BJERG, P. L., BOON, N., DEJONGHE, W., HUANG, W. E., SCHMIDT, S. I., SMOLDERS, E., SØRENSEN, S. R., SPRINGAEL, D. & BREUKELEN, B. M. V. 2015. Biodegradation: Updating the Concepts of Control for Microbial Cleanup in Contaminated Aquifers. *Environ sci technol*, 49, 7073-7081.
- MOR, S., RAVINDRA, K., DAHIYA, R. P. & CHANDRA, A. 2006. Leachate characterization and assessment of groundwater pollution near municipal solid waste landfill site. *Environ Monit Assess*, 118, 435-456.
- MULLER, A. K., WESTERGAARD, K., CHRISTENSEN, S. & SORENSON, S. J. 2001. The effect of long-term mercury pollution on the soil microbial community. *FEMS Microbiol Ecol*, 36, 11-19.
- PRUESSE, E., QUAST, C., KNITTEL, K., FUCHS, B. M., LUDWIG, W., PEPLIES, J. & GLOCKNER, F. O. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, 35, 7188-7196.
- RODRIGUES, V. D., TORRES, T. T. & OTTOBONI, L. M. 2014. Bacterial diversity assessment in soil of an active Brazilian copper mine using high-throughput sequencing of 16S rDNA amplicons. *Anton Van Leeuw*, 106, 879-90.
- ROLING, W. F. M., VAN BREUKELEN, B. M., BRASTER, M., LIN, B. & VAN VERSEVELD, H. W. 2001. Relationships between Microbial Community Structure and Hydrochemistry in a Landfill Leachate-Polluted Aquifer. *Appl Environ Microbiol*, 67, 4619-4629.

- 476 RONG, F., ZHAOGUI, G. & TUGEN, F. 2011. Analysis of Stability and Control in Landfill Sites Expansion.
477 *Proc Eng*, 24, 667-671.
- 478 SANDAA, R.-A., TORSVIK, V., ENGER, O., DAAE, F. L., CASTBERG, T. & HAHN, D. 1999. Analysis of bacterial
479 communities in heavy metal contaminated soils at different levels of resolution. *FEMS Microbiol*
480 *Ecol*, 30, 237-251.
- 481 SCHMIDTOVA, J. & BALDWIN, S. A. 2011. Correlation of bacterial communities supported by different
482 organic materials with sulfate reduction in metal-rich landfill leachate. *Water res*, 45, 1115-
483 1128.
- 484 SHI, X., FANG, R., WU, J., XU, H., SUN, Y. & YU, J. 2012. Sustainable development and utilization of
485 groundwater resources considering land subsidence in Suzhou, China. *Eng geol*, 124, 77-89.
- 486 SONG, L., WANG, Y., TANG, W. & LE, Y. 2015a. Bacterial community diversity in municipal waste landfill
487 sites. *Appl Microbiol Biotechnol*, 99, 7745-7756.
- 488 SONG, L., WANG, Y., ZHAO, H. & LONG, D. T. 2015b. Composition of bacterial and archaeal communities
489 during landfill refuse decomposition processes. *Microbiol res*, 181, 105-111.
- 490 STALEY, B. F., SAIKALY, P. E., DE LOS REYES, F. L., 3RD & BARLAZ, M. A. 2011. Critical evaluation of solid
491 waste sample processing for DNA-based microbial community analysis. *Biodegradation*, 22, 189-
492 204.
- 493 SULTANA, M., MOU, T. J., SANYAL, S. K., DIBA, F., MAHMUD, Z. H., PARVEZ, A. K. & HOSSAIN, M. A. 2017.
494 Investigation of Arsenotrophic Microbiome in Arsenic-Affected Bangladesh Groundwater.
495 *Ground Water*, 55, 736-746.
- 496 VISHNIVETSKAYA, T. A., MOSHER, J. J., PALUMBO, A. V., YANG, Z. K., PODAR, M., BROWN, S. D., BROOKS,
497 S. C., GU, B., SOUTHWORTH, G. R., DRAKE, M. M., BRANDT, C. C. & ELIAS, D. A. 2011. Mercury
498 and Other Heavy Metals Influence Bacterial Community Structure in Contaminated Tennessee
499 Streams. *Appl Environ Microbiol*, 77, 302-311.
- 500 VUKANTI, R., CRISSMAN, M., LEFF, L. G. & LEFF, A. A. 2009. Bacterial communities of tyre monofill sites:
501 growth on tyre shreds and leachate. *J Appl Microbiol*, 106, 1957-1966.
- 502 WAN, R., ZHANG, S. & XIE, S. 2012. Microbial community changes in aquifer sediment microcosm for
503 anaerobic anthracene biodegradation under methanogenic condition. *J Environ sci*, 24, 1498-
504 1503.
- 505 WANG, J., LIN, Z., LIN, K., WANG, C., ZHANG, W., CUI, C., LIN, J., DONG, Q. & HUANG, C. 2011.
506 Polybrominated diphenyl ethers in water, sediment, soil, and biological samples from different
507 industrial areas in Zhejiang, China. *J Haz Mat*, 197, 211-219.
- 508 WANG, X., CAO, A., ZHAO, G., ZHOU, C. & XU, R. 2017. Microbial community structure and diversity in a
509 municipal solid waste landfill. *Waste Manage*, 66, 79-87.
- 510 WIJESEKARA, S. S. R. M. D. H. R., SIRIWARDANA, A. R., KAWAMOTO, K., SILVA, N. D., MAYAKADUWA, S.
511 S., BASNAYAKE, B. F. A. & VITHANAGE, M. 2014. Fate and transport of pollutants through a
512 municipal solid waste landfill leachate in Sri Lanka. *Environ Earth Sci*, 72, 1707-1719.
- 513 XIE, B., XIONG, S., LIANG, S., HU, C., ZHANG, X. & LU, J. 2012. Performance and bacterial compositions of
514 aged refuse reactors treating mature landfill leachate. *Biores Technol*, 103, 71-77.
- 515 YANG, K., ZHOU, X.-N., YAN, W.-A., HANG, D.-R. & STEINMANN, P. 2008. Landfills in Jiangsu province,
516 China, and potential threats for public health: Leachate appraisal and spatial analysis using
517 geographic information system and remote sensing. *Waste Manage*, 28, 2750-2757.
- 518 YAO, X.-F., ZHANG, J.-M., TIAN, L. & GUO, J.-H. 2017. The effect of heavy metal contamination on the
519 bacterial community structure at Jiaozhou Bay, China. *Braz J Microbiol*, 48, 71-78.
- 520 ZHANG, H.-B., SHI, W., YANG, M.-X., SHA, T. & ZHAO, Z.-W. 2008. Bacterial Diversity at Different Depths
521 in Lead-Zinc Mine Tailings as Revealed by 16S rRNA Gene Libraries. *J Microbiol*, 45, 479-484.

- 522 ZHANG, Q. Q., TIAN, B. H., ZHANG, X., GHULAM, A., FANG, C. R. & HE, R. 2013a. Investigation on
523 characteristics of leachate and concentrated leachate in three landfill leachate treatment plants.
524 *Waste Manage*, 33, 2277-2286.
- 525 ZHANG, Q. Q., TIAN, B. H., ZHANG, X., GHULAM, A., FANG, C. R. & HE, R. 2013b. Investigation on
526 characteristics of leachate and concentrated leachate in three landfill leachate treatment plants.
527 *Waste Manag*, 33, 2277-86.
- 528 ZOU, J., DAI, W., GONG, S. & MA, Z. 2014. Analysis of Spatial Variations and Sources of Heavy Metals in
529 Farmland Soils of Beijing Suburbs. *PLOS one*, 10, 13.
- 530
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533 **Table captions:**

534 **Table 1.** Collection and description for landfill samples.

535 **Table 2.** pH and heavy metal composition in landfill leachate (RL & FL2) and ground water
536 samples (BHW) and urban site groundwater sample (USGW) respectively; ⁽¹⁾ represents the
537 first reading and ⁽²⁾ represents the second reading. ND = Not detected

538 **Table 3.** pH and heavy metal composition of samples obtained from landfill (LS1, LS2 & LS3)
539 and urban site (USS1 & USSUR1) soil respectively; ⁽¹⁾ represents the first reading and ⁽²⁾
540 represents the second reading. ND = Not detected

541 **Table 4.** Bacterial diversity based on 16S rRNA gene retrieved by NGS from a landfill and an
542 urban site. ACE = Abundance based coverage estimators

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Figure captions:

Fig 1. Phylum level bacterial community composition observed in the samples collected from landfill site (a) and an urban site (b). FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW = landfill ground water; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples.

Fig 2. Bacterial community composition and cluster analysis at order level in samples collected from landfill site and an urban site. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil locations; BHGW = landfill ground water; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples.

Fig 3. Genus level bacterial community composition observed in the samples collected from landfill site (a) and an urban site (b). FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW= landfill ground water; USGW= urban site ground water; USS1 and USSUR1= urban site soil samples.

Fig 4. Cluster analysis based on order level bacterial abundance. (a) LEA, USO, LSO; (b) GW, LEA, LSO. FL2=fresh leachate; RL= raw leachate; LS1, LS2 and LS3 = landfill soil; BHGW= landfill ground water; USGW= urban site ground water; USS1 and USSUR1= urban site soil samples; GW=combination of groundwater from both sites.

Fig 5. Non-metric multidimensional scaling (NMDS) analysis of sequences. (a) LF and US; (b) LEA, LSO, USO. FL2 = fresh leachate; RL = raw leachate; LS1, LS2 and LS3 = landfill soil locations; BHGW = landfill ground water; LF = combination of all landfill samples; USGW = urban site ground water; USS1 and USSUR1 = urban site soil samples; US = combination of all urban sites.

Fig 6. Redundancy analysis (RDA) of soil bacterial communities in landfill and urban site soil samples. RDA1 explained 89.2 %, and RDA2 explained 7.65 % of the total variance. LS1, LS2 and LS3 = landfill soil locations USS1 and USSUR1 = urban site soil samples , respectively

573 **Fig 7.** Canonical correspondence analysis (CCA) of bacterial communities in RL, FL2, BHGW
574 and USGW. CCA1 explained 49.01 %, and CCA2 explained 45.97 % of the total variance. FL2
575 = fresh leachate; RL = raw leachate; BHGW = landfill ground water; USGW = urban site ground
576 water, respectively.

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Table 1

Samples acronyms	Sample name	Reason for collection
RL	Raw Leachate	Due to its long term storage in the landfill that might influence variation in the microbial diversity.
FL2	Fresh Leachate	Provides an in depth understanding on the microbial diversity when compared with raw leachate
LS1	Landfill soil location 1	Closer to the landfill which might provide data on any leakage from leachate.
LS2	Landfill soil location 2	Closer to the agricultural land; data can be used to compare with landfill soil location 1.
LS3	Landfill soil location 3	Closer to the groundwater monitoring borehole; data can be used to compare the permeability of the landfill.
BHGW	Landfill groundwater monitoring borehole	Only functioning borehole used to check the contamination levels of the groundwater.
USGW	Urban site groundwater	Accessible borehole close to the soil locations.
USS1	Urban site soil sample 1	Location of the soil sampling was located closer to the urban site groundwater. It was collected from the surface.
USSur1	Urban site soil sample 2	Isolated soil location 500 m away from USS1 and USGW. It was collected 30 cm depth.

Table 2

	pH	Mercury (µg/L)	Arsenic (µg/L)	Cadmium (µg/L)	Copper (µg/L)	Lead (µg/L)	Zinc (µg/L)	Chromium (µg/L)
RL ¹	7.78	10.9	1.11x10 ³	ND	ND	ND	ND	0.508
RL ²	7.9	11.42	1.23x10 ³	ND	ND	ND	ND	0.581
FL2¹	8.12	7.37	1.78x10 ³	ND	0.107	0.027	ND	0.586
FL2 ²	8.3	8.20	1.84x10 ³	ND	ND	ND	ND	0.541
BHGW ¹	8.2	12.7	ND	ND	0.048	ND	0.186	0.015
BHGW ²	8.25	5.59	ND	ND	ND	ND	0.062	0.011
USGW	7.75	0.037	ND	ND	ND	0.078	0.030	ND

Table 3

	pH	Mercury	Arsenic	Cadmium	Copper	Lead	Zinc	Chromium
		(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
LS1 ¹	6.71	0.175	0.766	ND	69.5	10.3	49.1	62.3
LS1 ²	6.87	0.152	0.854	ND	75.3	10.1	81.4	67.3
LS2 ¹	6.63	0.150	0.937	ND	79.3	5.72	55.7	70.4
LS2 ²	6.42	0.184	0.726	ND	77.2	7.90	71.2	71.5
LS3 ¹	7.1	0.146	0.998	ND	79.5	6.91	76.9	73.8
LS3 ²	6.95	0.143	0.907	ND	71.8	8.73	64.8	68.2
USS1	6.82	0.075	11.3	0.169	5.8	27.6	64.9	43.45
USSUR1	6.74	0.058	9.28	0.137	7.57	26.3	63.6	50.2

Table 4

Sample ID	Number of Reads	Number of OTUs	ACE index	Chao 1 richness estimate	Shannon diversity index (H')	Simpson diversity index (D)	Coverage
					0.97		
RL	15386	154	159	164	2.06	0.3716	0.999
FL2	15746	139	174	163	0.98	0.6584	0.997
LS1	15313	996	1109	1103	5.77	0.0089	0.989
LS2	13611	647	892	862	3.43	0.125	0.983
LS3	20464	875	1080	1112	4.49	0.0516	0.989
BHGW	20141	1018	1201	1259	5.6	0.0093	0.988
USGW	22643	168	189	190	2.65	0.177	0.999
USS1	16625	1224	1331	1332	6.1	0.0056	0.989
USSUR1	14015	1167	1322	1328	5.94	0.0079	0.983

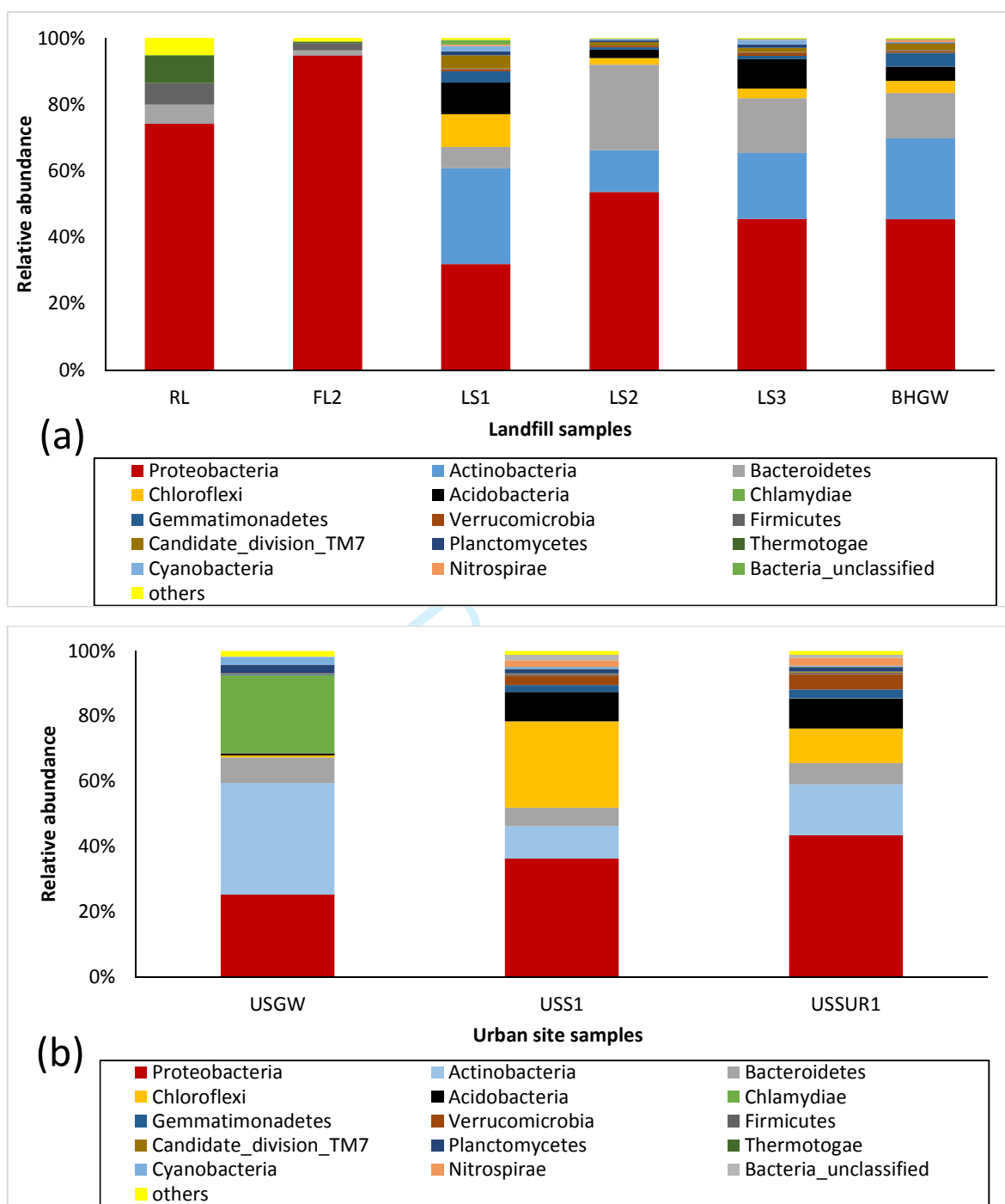


Fig 1.

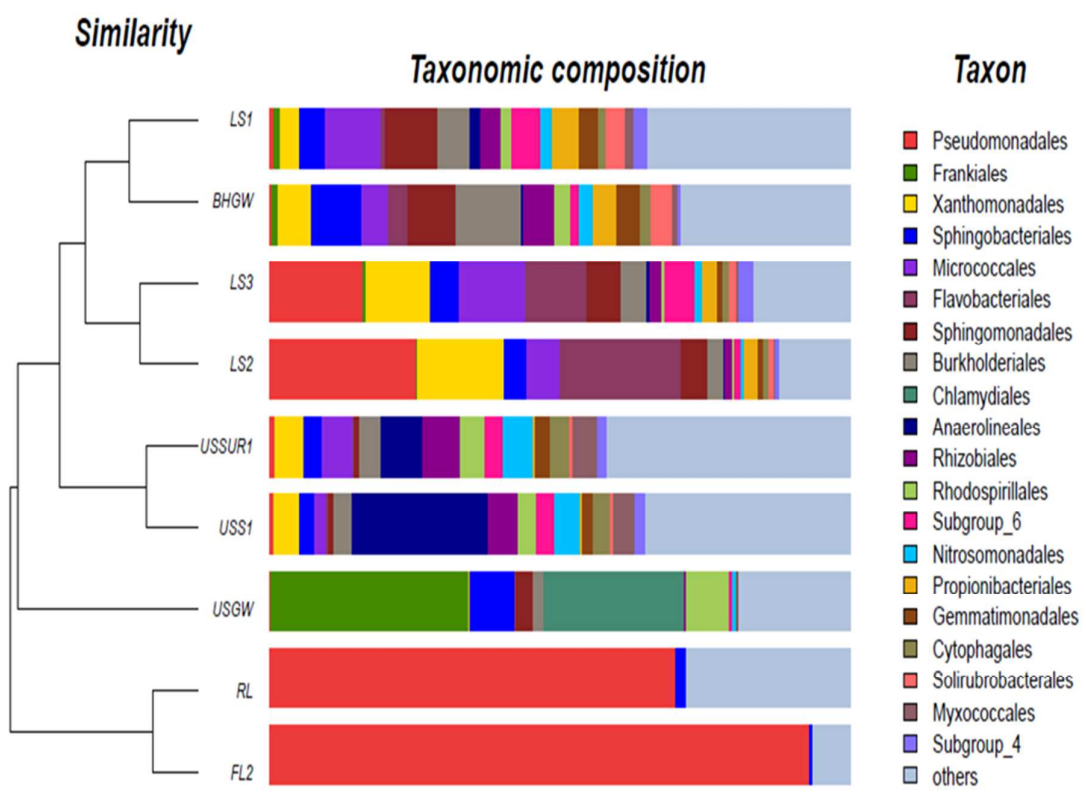


Fig 2.

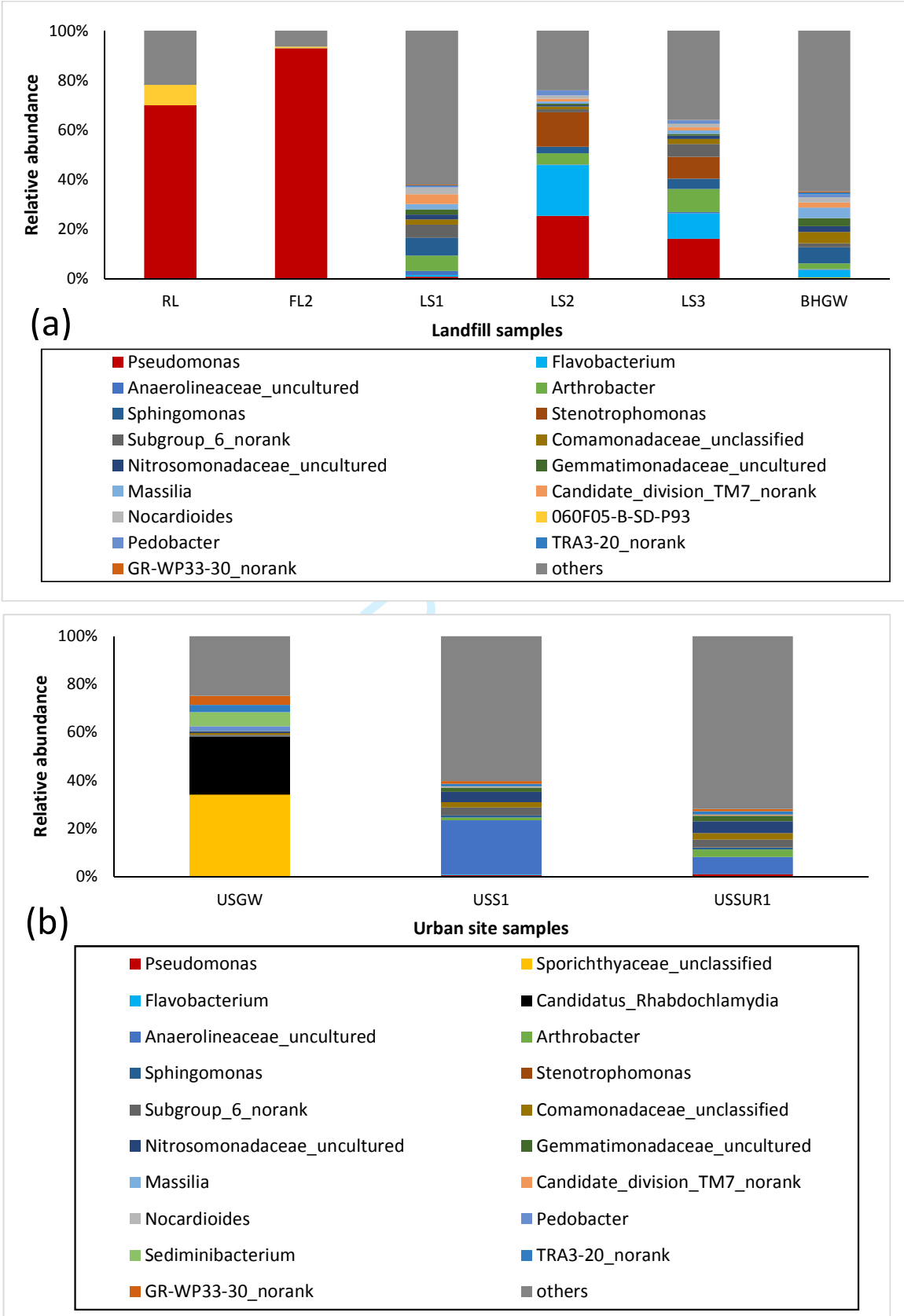
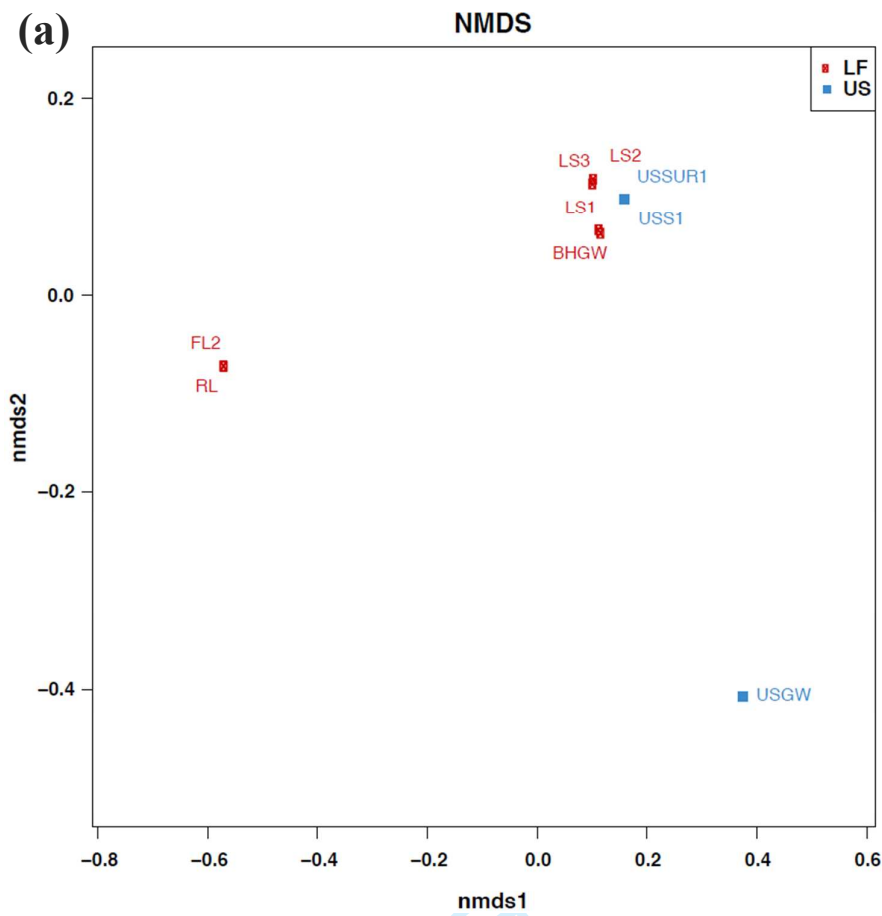


Fig 3.



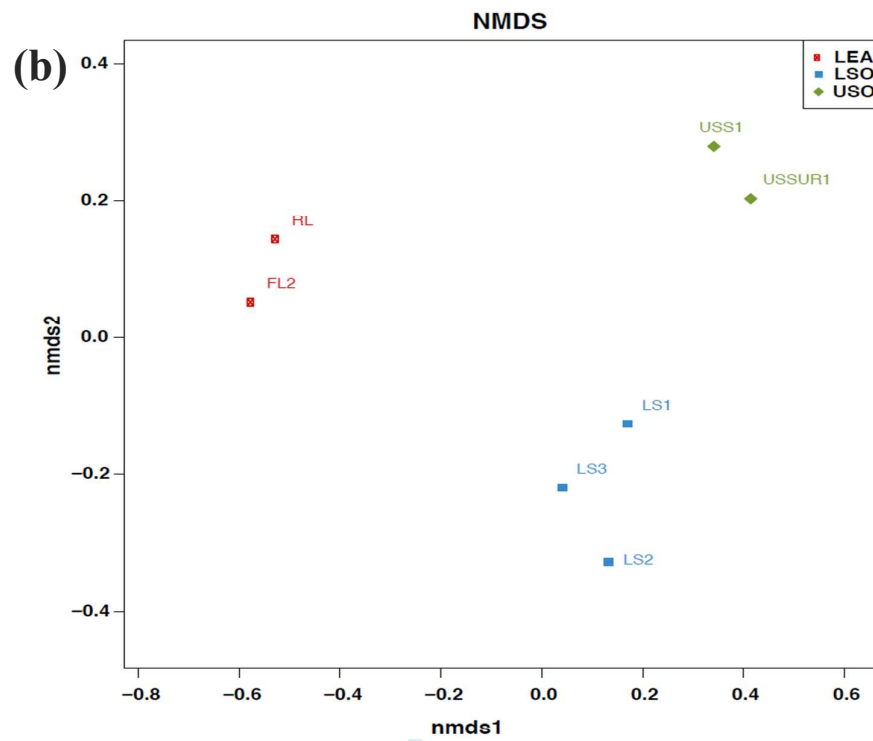


Fig 4.

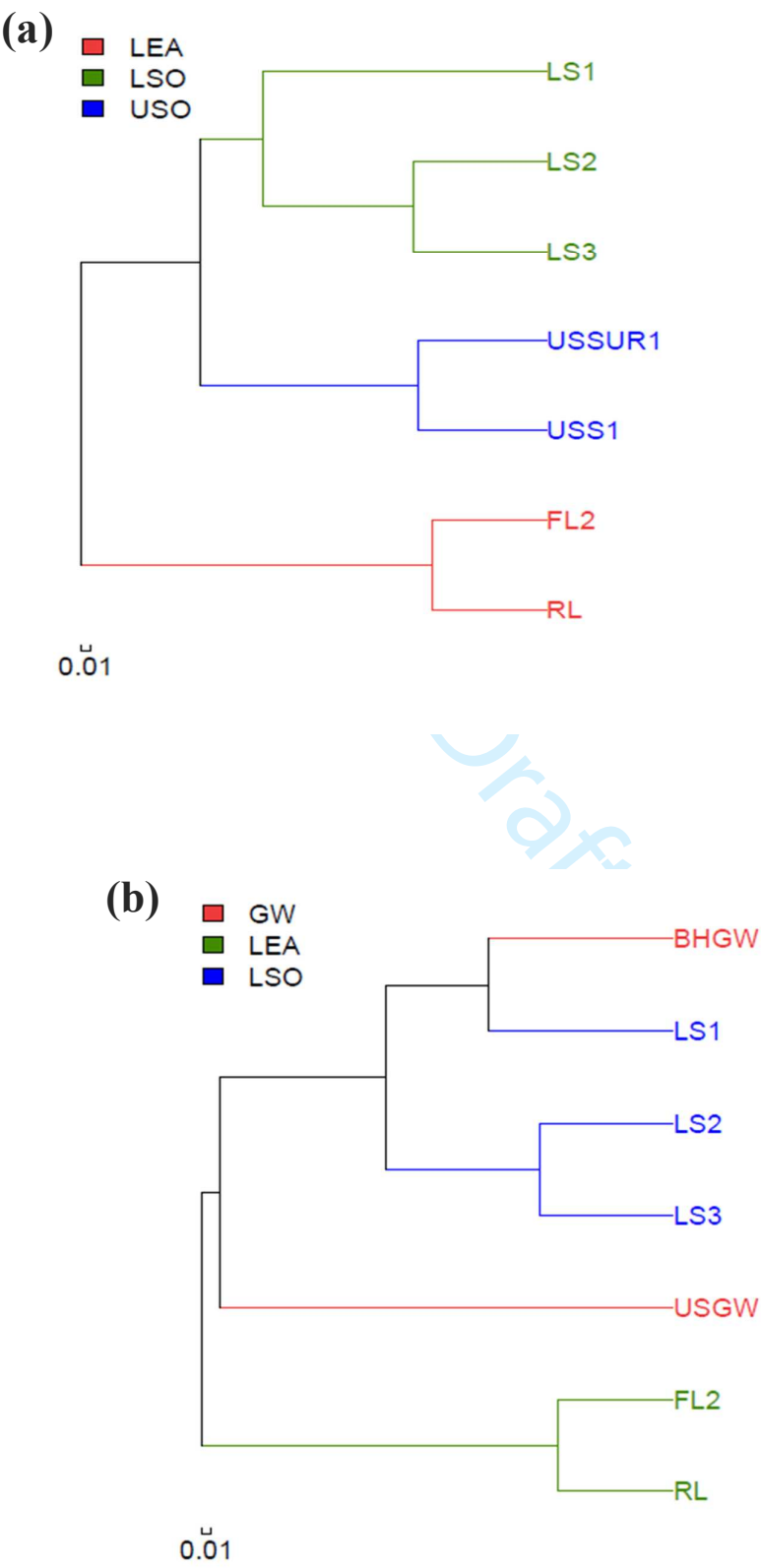


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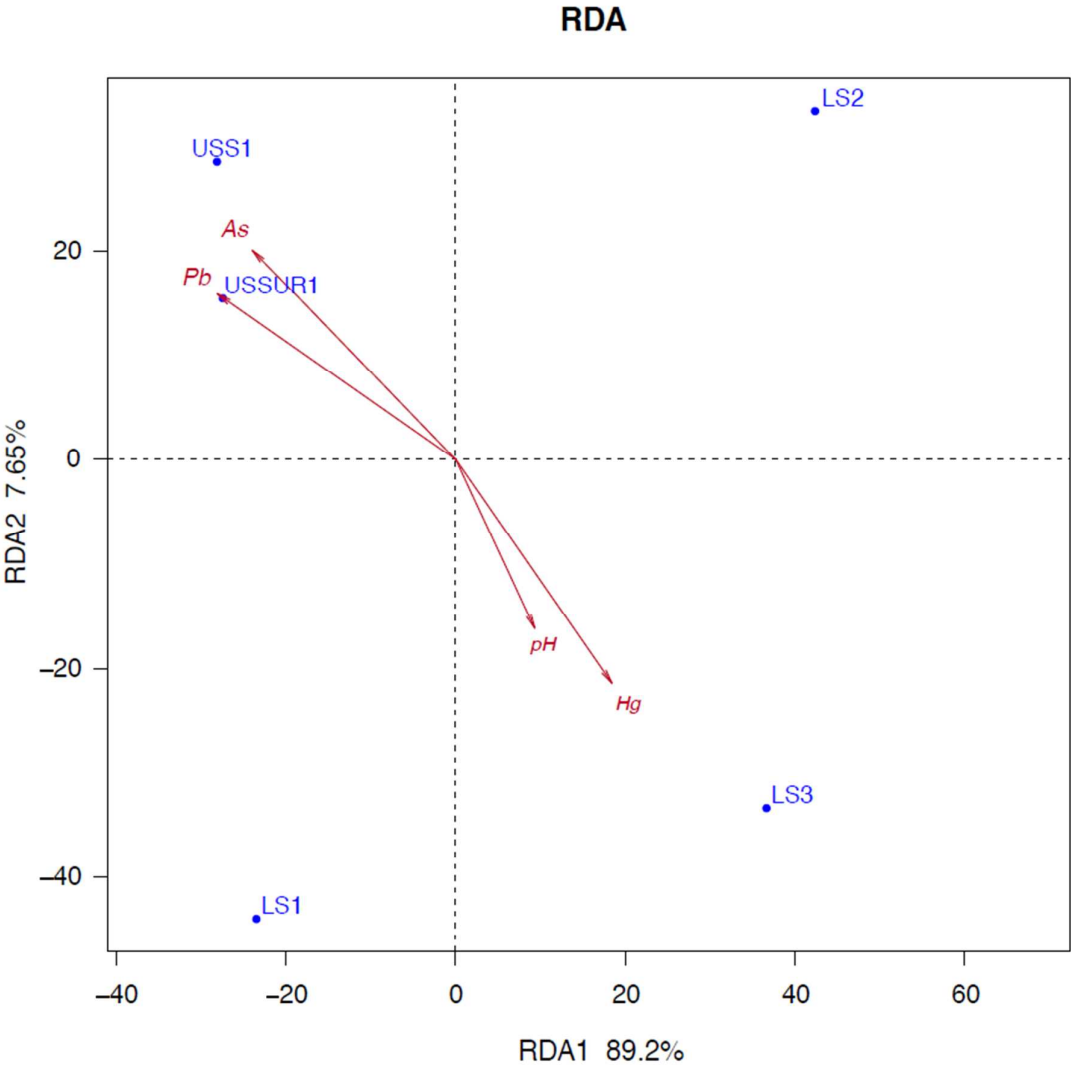


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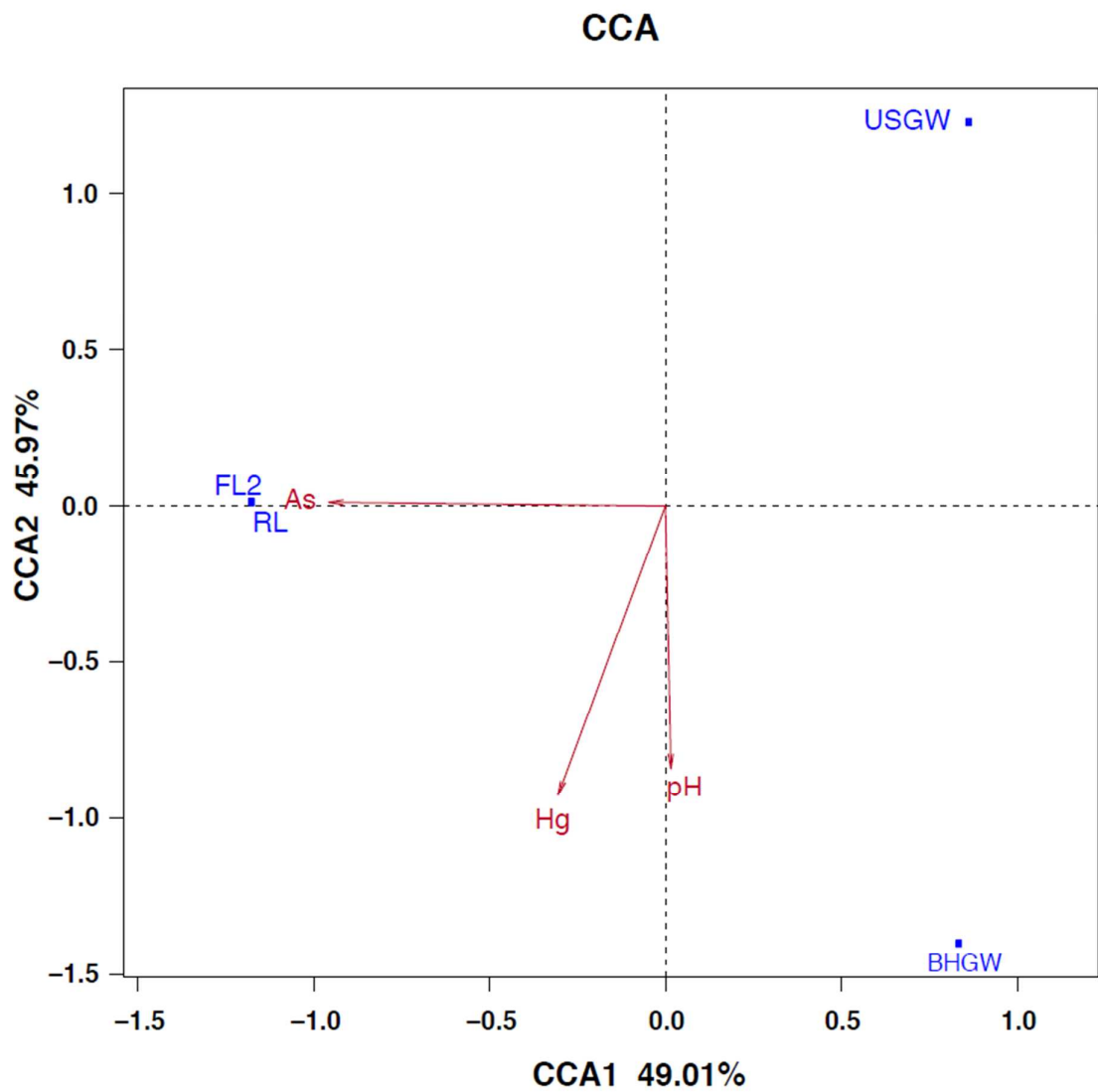


Fig 7.